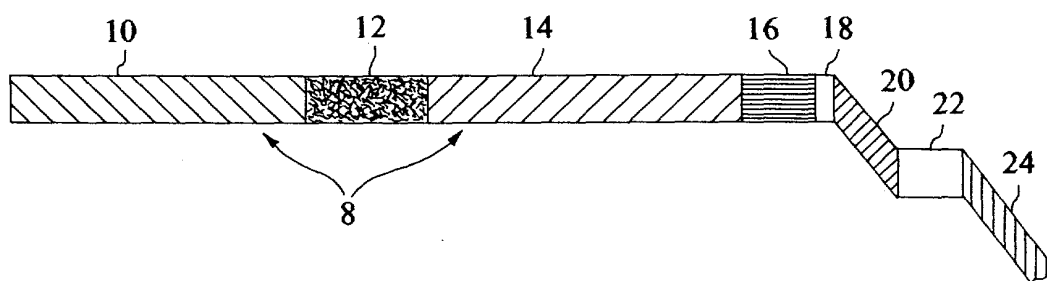
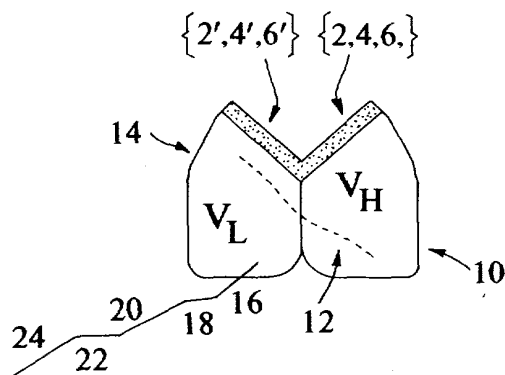


SINGLE CHAIN BINDING POLYPEPTIDE



Extended Polypeptide

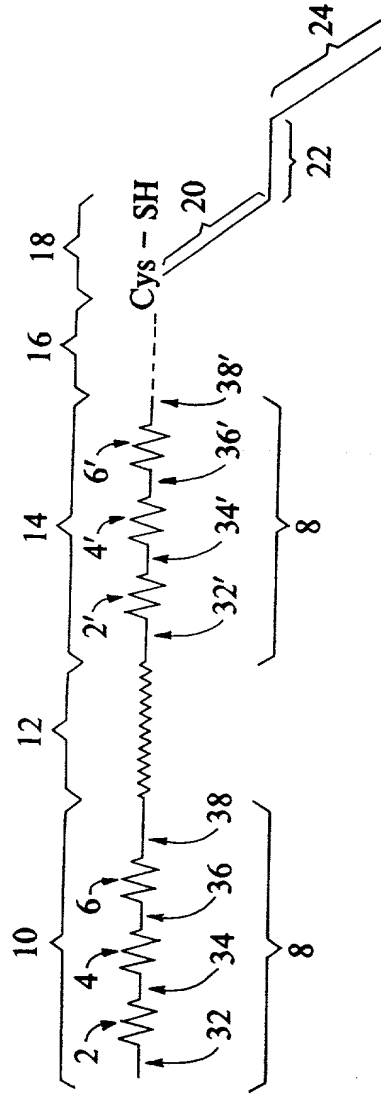
FIG. 1(a)



Folded Protein

FIG. 1(b)

SINGLE CHAIN
BINDING POLYPEPTIDE SHOWING
LOCATIONS OF COMPLEMENTARITY
DETERMINING REGIONS, POLYPEPTIDE
SPACER REGIONS, AND EFFECTOR REGIONS



**C6.5 sFv
AMINO ACID SEQUENCE**

(N-terminus to C-terminus)

-QVQLQSGAELKKPGESLKISCKGSGYSFTSYIAWVROMPGKGLEVMGL
IYPGDSDTKYSPSFQGVTTISVDKSVSTAYLQWSSLKPSDSAIFYCARHD
VGYCSSNCAKWPEYFQHWGQGLTVTVSSGGGSGGGSG
GGGSQSVLTQPPSVSAAPGQKVTISCSGSSNIGNNYVSVYQQLPCTAPK
LLIYHTNRPAGVPDRFSGSKGTSASLAISGRSEADYYCAAWDDSL
SGWVFGGGTKLTVLG

FIG. 3

**C6.5 sFv
NUCLEOTIDE SEQUENCE**

5'caggtgcagctgttgaggcagagttgaaaaaacccggggagtcctgaagatcctctgaagggttctggataca
gccttaccagctactggatcgccctgggtgcggccagatgcccggggaaaggcctggagtagacatggggctcatctatcctggtgactc
tgacaccaaatatacagcccgctctccaaaggccagggtcaccatctcagtcagacaagtcgcgtcagcactgcctacttgcaatggagc
agtcagaagccctcggacagcgccgtgtatttttggcgagacatgaagtgaggatattgcagtagttccaaactgcgcaaatggcc
tgaatacttccagcattggggcccaaggcaccctggtcaccgtctcctcaggtggagcggttcaggcgagggtggctctggcg
gtggcggatcgagctctgtgttgacgcagccgcccctcagtgctgtggggcccccaggacagaaggtcaccatctcctgctctggaa
gcagctccaacattgggaataattatgtatcctgggtaccagcagctccccagggaacagccccaaactctcatctatggtcacacca
atcggcccgaggggtccctgaccgattctctggctccaaagtctggcacctcagcctccctggccatcagtggggttccgggtccga
ggatgagggtgattattactgtgcagcatgggatgacagcctgagtggtgggtgttcggcggaggggaccaagctgaccgtcct
aggt 3'

FIG. 4

COML3-9 sFv'
AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAEVKKPGESLKISKSGSYFTSYWIAWVRQMPKGLEYMGL
IYPGDSDTKYSPSFQGGVTISVDKSVSTAYLQWSSLPKPSDSAVYFCARHD
VGYCSSNCAKWPYFQHWGQGLTVTVSSGGGGSGGGSG
GGGSQSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNVYSWYQLPGTAPK
LLIYDHTNRPAGVPDRFSGSKSGTSASLAISGRSEDEADYYCASWDYTL
SGWVFGGCTKLTVLGAHHHHHHGGGGG-

FIG. 5

COML3-9 sFv'
NUCLEOTIDE SEQUENCE

5' caggcgagctggcagctctggggcagagggtgaaagccccggggagtgctctgaagatctcctgtaagggctctggata
cagctttaccagctactggatcgccctgggtgccccagatgccgggaaaggcctggagtagatggggctcatctatcctg
gtgactctgacaccaaatacagccccctcttccaggccagggtcaccatctcagtcgacaaagtcgcgactgcctac
ttgcaatggagcagctctgaagccctcgacagccgtgtattttgtgagagacatgacgtggggatattgcagtagttc
caactgcgcaaatggccctgaatacttccagcattggggccaggccaccctgggtcacccgtctcctcaggtggagggcggtt
caggcggagggtggctctggcggggggatcgccagctctgtgttgacgcagcccccctcagtgctcgggccccaggacag
aaggctaccatctcctgctctgggaagcagctcccaacattgggaataaattatgtatcctggtagccagcagctccagggaac
agccccaaactcctcatctatgatcacaccaatcgccccagggggtccctgaccgattctctggctcccaagtctggca
cctcagcctccctggccatcagtggggttccgggtccgaggatgaggctgattattactgtgcctcctgggactacacccctc
tcgggctgggtgttcggcgagggaaccaaagctgaccgtcttaggtgcggccgcacaccatcatcacgggtgggtgg
cggctgc 3'

FIG. 6

**C6ML3-9 sFv'-L1-KDEL
AMINO ACID SEQUENCE**

(N-terminus to C-terminus)

-QVLVQSGAEVKKPGESLKISKGSGYSTSYWIAWVRQMPKGLEYMGL
IYPGSDTKYSPFQGVVTSVDKSVSTAYLQWSSLKPSDSAIFYCARHD
VGYCSSNCAKWPEYFQHWGQCTLVTVSSGGGSGGGSG
GGGSQSVLTQPPSVSAAPGQKVTISCSGSSNIGNNYVSWYQLPGTAPK
LLIYDHTNRPAGVPDRFSGSKSGTSASLAISGFRSEDEADYICASWDYTL
SGWVFGGCTKLTVLGAAHHHHHHGGGCGLESSSGSEKDEL

FIG. 7

**C6ML3-9 sFv'-L1-KDEL
NUCLEOTIDE SEQUENCE**

5' caggtgcagctggtgcagctctggggcagaggtgaaaagccggggagtcctgaagatctcctgaagggtcttgata
cagctttaccagctactggatcgccctgggtgcgccagatgccgggaaagcctggagtagatggggctcatctatcctg
gtgactctgacaccaaatacacagcccgctcctccaaaggccaggtcaccatctcagtcgacaaagtcctcagcactgcctac
ttgcaatggagcagctctgaagccctcggacagcgccgtgtatttttgcgagagacatgacgtgggatatgtagtagttc
caactgcgcaaaagtggcctgaatacttccagcattggggccagggcaccctggtcaccgtctcctcaggtggaggggtt
caggcggaggtggctctggcgggtggcggatcgagctctgtgtgacgcagccgccctcagtgctctggggccccaggacag
aaggtcaccatctcctgctctggaaagcagctcccaattgggaataattatgtatcctgggtaccagcagctcccagggaac
agcccccaactcctcatctatgatcacaccaaatcgggcccgaggggtccctgaccgattctctggtcccaagctctggca
cctcagcctccctggccatcagtggggttcgggtccgggtcggaggtgaggtgattattactgtgctcctgggagctacaccctc
tcgggtgggtgttcggcggaggaaccaagctgaccgtccttaggtggcggccgcacaccatcatcaccatcacgggtgggtgg
cggctgcctcgagtcctctagctctggatccgaaaaagatgaactg 3'

FIG. 8

**C6ML3-9 sFv' -L2-KDEL
AMINO ACID SEQUENCE**

(N-terminus to C-terminus)

-QVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIAVVRQMPGKLEYMGL
IYPGDSDTKYSPSFQGVITISVDKSVSTAYLQWSSLPKPSDSAVYFCARHD
VGYCSSNCAKWPYFQHWGQGLTVTVSSGGGGGGGGGG
GGGSQSVLTQPPSVSAAPGQKVITISCGSSSNNIGNNYVSWYQQLPGTAPK
LLIYDHTNRPAGVDFRSGSKSGTSLAISGRSEDEADYYCASWDYTL
SGWVFGGGLTLVLGAAHHHHHHGGGGCLESSESSSSSSSGSEKDEL-

FIG. 9

**C6ML3-9sFv' -L2-KEDL
NUCLEOTIDE SEQUENCE**

5' cagggtgcagctgggtgcagctctgggggcagaggtagaaaaagccggggagtcctctgaagatctcctgtaagggttctggata
cagctttaccagctactggatgcctgggtgcgccagatgcccggggaaaggccctggagtagacatggggctcattctcctg
gtgactctgacaccaaatacagcccgctccttccaaggccagggtcaccatctcagtcgacacagtcgcactgcctac
ttgcaatggagcagctgaagccctcggacagcgcgtgtattttgtcgagacatgacgtgggatatattgcagtagttc
caactgcggcaagtggcctgaatacttcagcattggggccaggccaccctggtcaccgtctcctcaggtggaggcgggtt
caggcggagggtggctctggcgggaggcggatcgccagctgtgtgtgacgcagccggccctcagtgctgcggcccccaggacag
aaggtcaccatctcctgctctgggaagcagctccaacattggggaataattatgtatcctgggtaccagcagctcccagggaac
agcccccaactcctcatctatgatcacaccaatcgccccgagggtccctgaccgattctctggctccaagtctggca
cctcagcctccctggccatcagtggggttcgggtccgaggatgaggctgattactgtgctcctcctgggactacacccctc
tcggggctgggtgttcggcggagggaaccaaagctgaccgtcctagggtgcggccgcacaccatcaccatcagggtgggtgg
cggctgcctcaggtctagcagctccgggttcctctagctctggatccgaaaaaatgaactg 3'

FIG. 10

**C6ML3-9 sFv' -L2-H14
AMINO ACID SEQUENCE**

(N-terminus to C-terminus)

-QVQLVQSGAEVKKPESLKISCKGSGYSFTSYWIAWVRQMPKGLEYMGL
IYPGDSDTKYSPSFQGVVTSVDKSVSTAYLQWSSLKPSDSAVYFCARHD
VGYCSSNCAKWPEYFQHWGQGLTVTVSSGGGGSGGGSG
GGGSQSVLTQPPSVSAAPGQKVTISCSGSSNIGNNYVSWYQQLPGTAPK
LLIYDHTNRPAGVPDRFSGSKSGTSASLAISGRSEADYYCASWDYTL
SGWVFGGCTKLTVLGAHHHHHHGGGGGLESSSSSGSSSS
GSKSAKTPKAKKP-

FIG. 11

**C6ML3-9 sFv' -L2-H14
NUCLEOTIDE SEQUENCE**

5' cagggtgcagctgggtgcagctctggggcagaggtgaaaaagcccggggagtcctctgaagatctcctgtaagggttctggata
cagctttaccagctactggatgggtgcggccagatgcccggggaaaggcctggagtacatggggctcatctatcctg
gtgactctgacaccaaataacagcccgctccttccaaggccaggtcaccatctcagtcgacagtcctgcgcagcactgcctac
ttgcaatggagcagctgaagccctcggacagcgccgtgtatttttggcgagacatgacgtgggatatattgcagtagttc
caactgcgcaaatggcctgatacttccagcattggggccagggcacccctggtcaccgtctcctcaggtggagggcgggtt
caggcggaggtggcctctggcgggtggcggatcgccagctctgtgttgacgcagccgcccctcagtgctgcggcccccaggacag
aaggtcaccatctcctgctctgggaagcagctcccaacattggggaataattatgtatcctgggtaccagcagctcccaggaaac
agcccccaactcctcatctatgatcacaccaatcgccccgcaggggtccctgaccgattctctggctcccaagctctggca
cctcagcctccctggccatcagtggggttcgggtccgaggatgaggctgattattactgtgctcctctgggactacacccctc
tcgggtgggtgttcggcggaggaaacaaagctgacgcgtccttaggtgcggccgcacaccatcatcaccatcaggtgggtgg
cggctgcctcgagcttagcagctccgggttcctcttagctctggatcccaagaaagcgcgaaaaagaccccgaaagaaag
cgaagaaacgg 3'

FIG. 12

C6ML3-9 sFv'-L2-nls
AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAEVKKPESLKISKCKSGYSTSYWIAWVRQMPGKGL
IYPGDSDTKYSPSFQGGVTISVDKSVSTAYLQWSSLPKPSDAVYFCARHD
VGYCSSNCAKWPYFQHWGQGTLVTVSSGGGSGGGGSG
GGGSQSVLTQPPSVSAAPGQKVITISCGSSSNIGNNYVSWYQQLPGTAPK
LLIYDHTNRPAGVPDRFSGSKSGTASLAISGRSEDEADYYCASWDYTL
SGWVFGGCTKLTVLGAHHHHHHGGGGGLESSSSSSSSSS
GSTPPKKKKKV

FIG. 13

C6ML3-9 sFv'-L2-nls
NUCLEOTIDE SEQUENCE

5' caggtgcagctggtgcagcttggggcagaggtgaaaaagccccggggaggtctctgaagatctctgtaagggttcttgata
cagctttaccagctactggatcgccctgggtggccagatgccccgggaaggcctggaggtacatggggctcatctatctctg
gtgactctgacaccaaatacagcccgctctcccaaggccaggtcaccatctcagtcgacaaagtcctgcagcactgcctac
ttgcaatggagcagtgaaagccctggacagcgccgtgtattttgtgcgagacatgacgtgggatatattgcagtagttc
caactgcgcaaaagtggcctgaatacttccagcattggggccagggcacccctggtcaccgtctctcctcaggtggaggcgggtt
caggcggagggtgggtcttggcggtaggcagtcgtgtgtgacgcagccgcccctcagtggtctgcggccccaggacag
aaggtcaccatctctctgttggaagcagctcccaacattgggaataattatgtatctctggtagcagcagctccaggaaac
agcccccaaaactctcatctatgatcacaccaatggccccgaggggtccctgaccgattctctggctccaaagtctggca
cctcagccctccctggccatcagtggggttccgggtccgaggtgaggtgattattactgtgctcctgggactacacccctc
tcgggctgggtgttcggcggagggaaccaagctgaccgtcctaggtgcggccgcacacccatcatcaccatcaggtgggtgg
cggctgcctcagctagcagctccgggttctcttagctctggatccactccgccgaaaaaagaacgtaaagtg 3'

FIG. 14

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C6ML3-9 sFv' and its salmon protamine conjugate binds specifically to the erbB-2 positive ovarian cancer cells

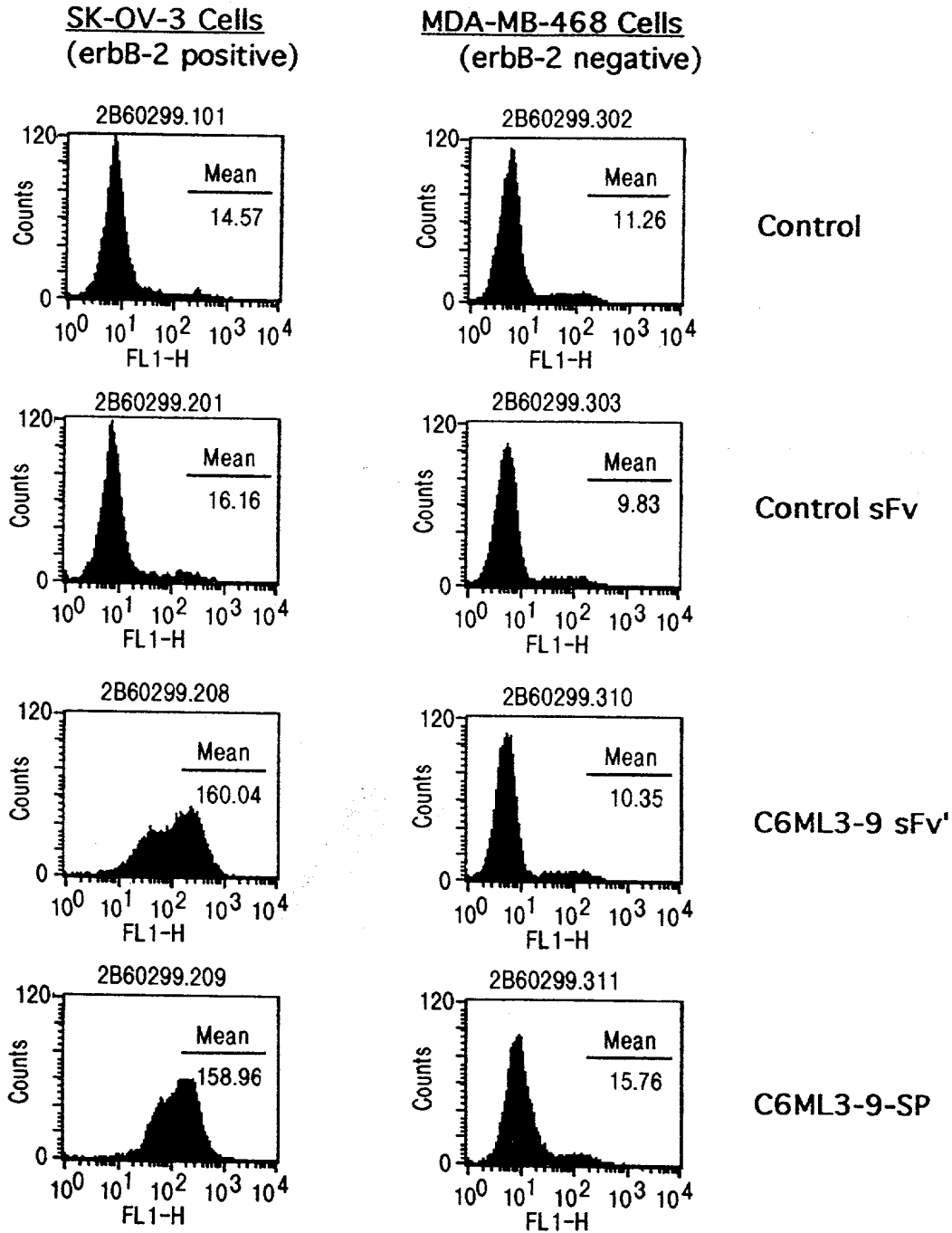


FIG. 15

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FACS Analysis of the erbB-2 Binding
Activities of Bacterially Expressed C6ML3-9 sFv'
and its Derivatives

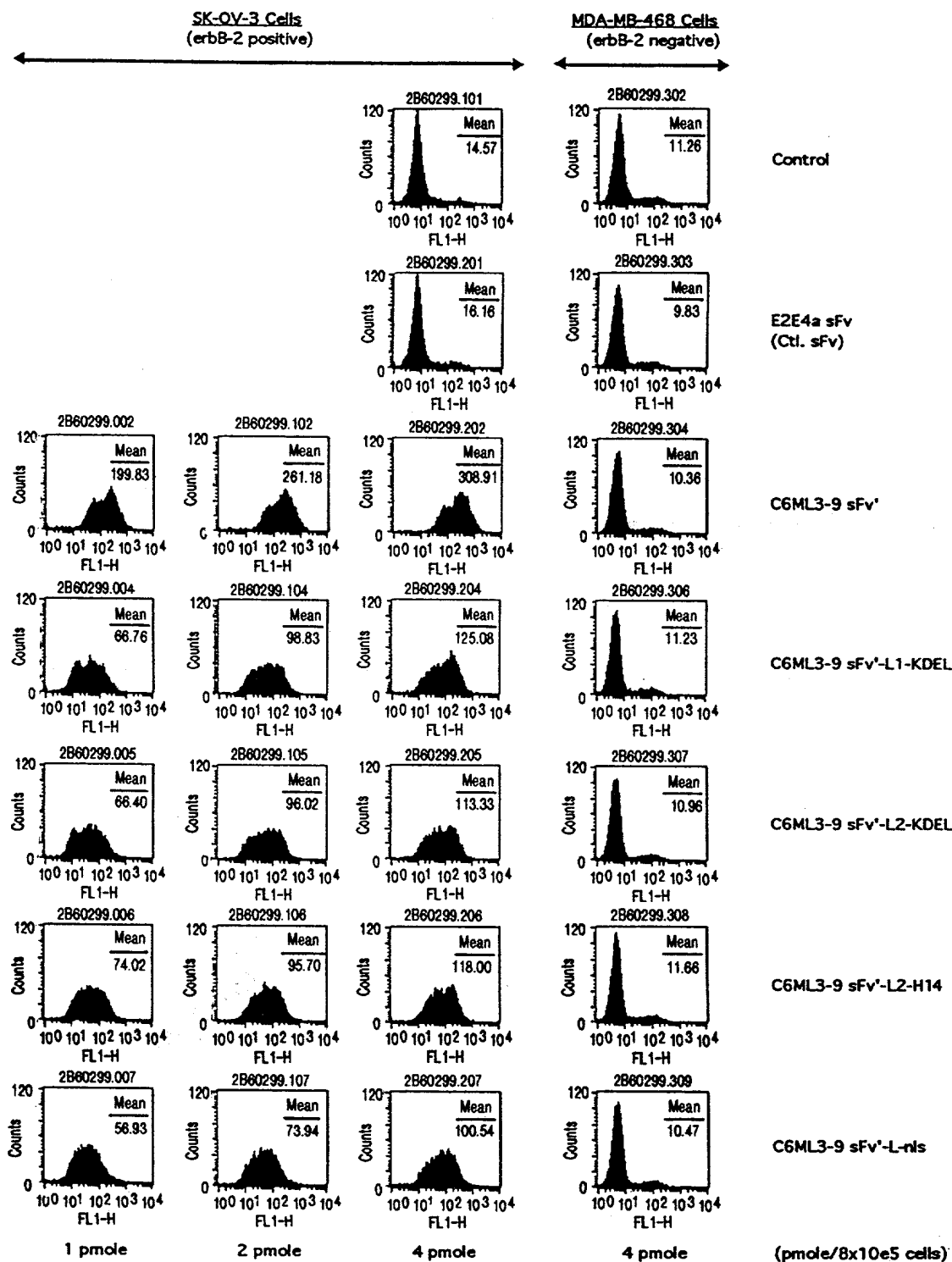
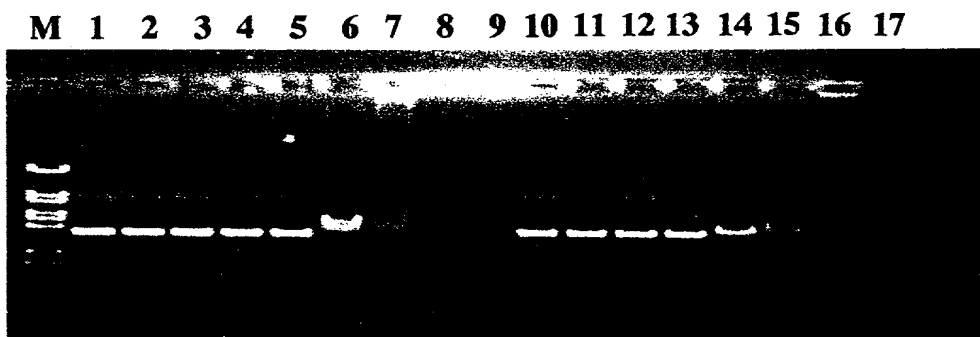


FIG. 16

Gel Shift Analysis of the C6.5-SP-DNA and C6ML3-9-SP-DNA Complex

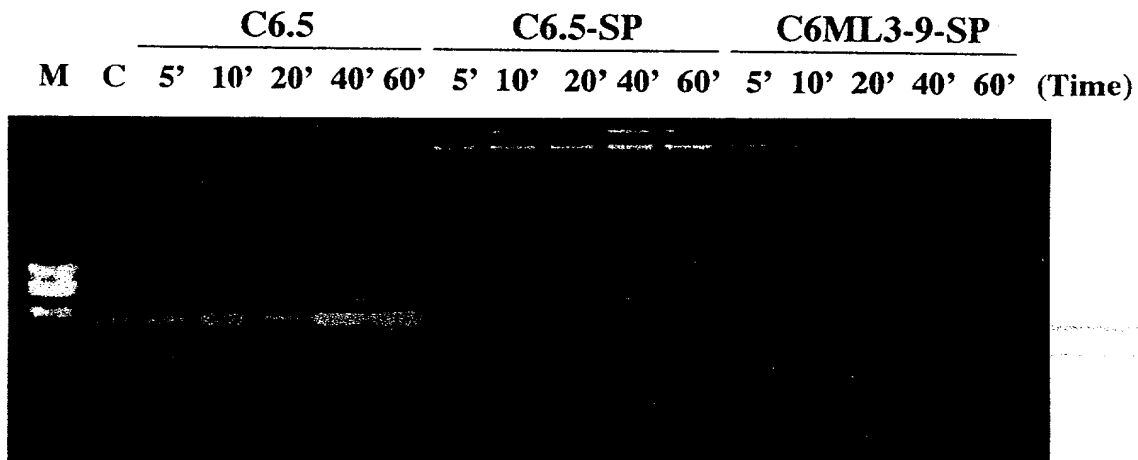


- M. DNA marker - λ DNA BstEII digest
1. 200 ng pGL3 DNA
 2. 200 ng pGL3 DNA + 1.45 pmol C6.5
 3. 200 ng pGL3 DNA + 2.90 pmol C6.5
 4. 200 ng pGL3 DNA + 5.80 pmol C6.5
 5. 200 ng pGL3 DNA + 11.6 pmol C6.5
 6. 200 ng pGL3 DNA + 1.45 pmol C6.5-SP
 7. 200 ng pGL3 DNA + 2.90 pmol C6.5-SP
 8. 200 ng pGL3 DNA + 5.80 pmol C6.5-SP
 9. 200 ng pGL3 DNA + 11.6 pmol C6.5-SP
 10. 200 ng pGL3 DNA + 1.45 pmol C6ML3-9
 11. 200 ng pGL3 DNA + 2.90 pmol C6ML3-9
 12. 200 ng pGL3 DNA + 5.80 pmol C6ML3-9
 13. 200 ng pGL3 DNA + 11.6 pmol C6ML3-9
 14. 200 ng pGL3 DNA + 1.45 pmol C6ML3-9-SP
 15. 200 ng pGL3 DNA + 2.90 pmol C6ML3-9-SP
 16. 200 ng pGL3 DNA + 5.80 pmol C6ML3-9-SP
 17. 200 ng pGL3 DNA + 11.6 pmol C6ML3-9-SP

*0.8% agarose gel in 1xTAE, 150v, RT, ~1hr, EtBr staining overnight

FIG. 17

Kinetic Study of the C6.5-SP-DNA and C6ML3-9-SP-DNA Complex Formation



M. DNA marker - λ DNA BstEII digest

C. 200 ng pGL3 DNA alone

* The rest of the lanes - 200 ng pGL3 DNA incubated with 5.8 pmol proteins as indicated above each line, on ice, for different period of time. Electrophoresis condition same as Figure 17.

FIG. 18

**The C6ML3-9-SP conjugate protein mediates
specific luciferase gene delivery to erbB-2 positive cancer cells**

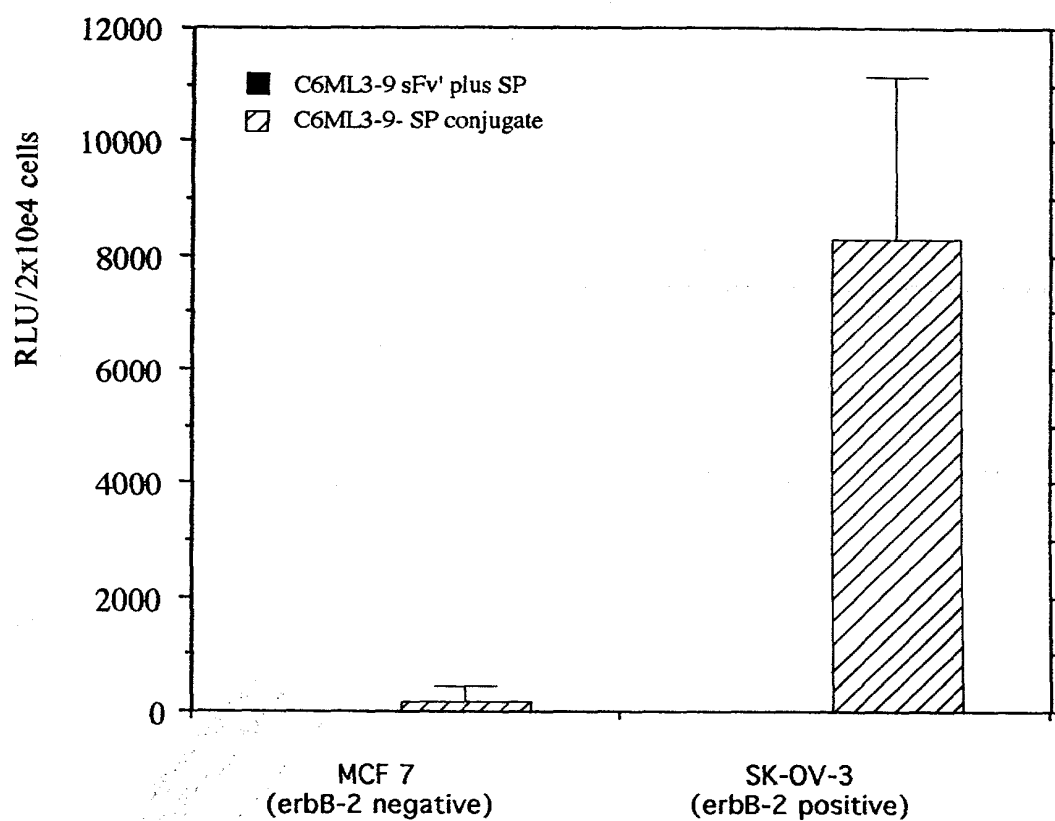
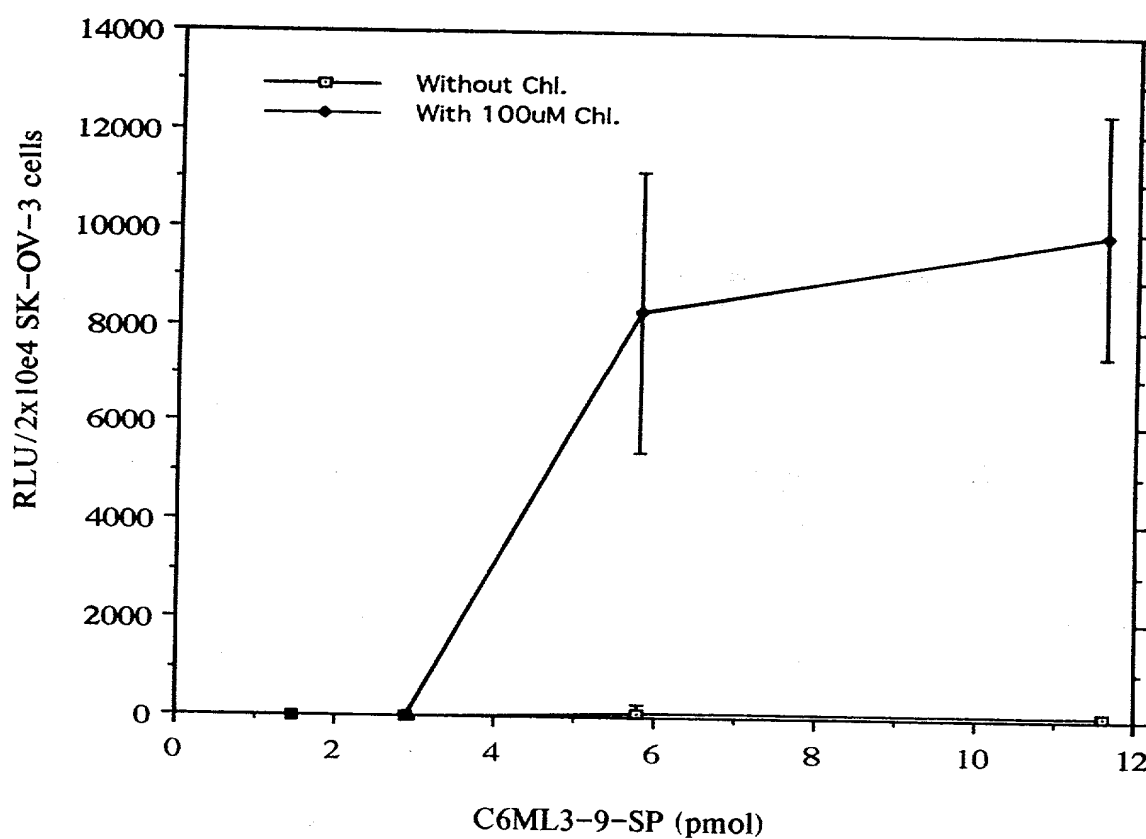


FIG. 19

Chloroquine-dependent C6ML3-9-SP-mediated Gene Delivery

**FIG. 20**

Fluorescent microscopy of C6.5-SP and C6ML3-9
-SP-mediated gene transfer of pGeneGrip Rhodamine/GFP
plasmids with SK-OV-3 and MCF-7

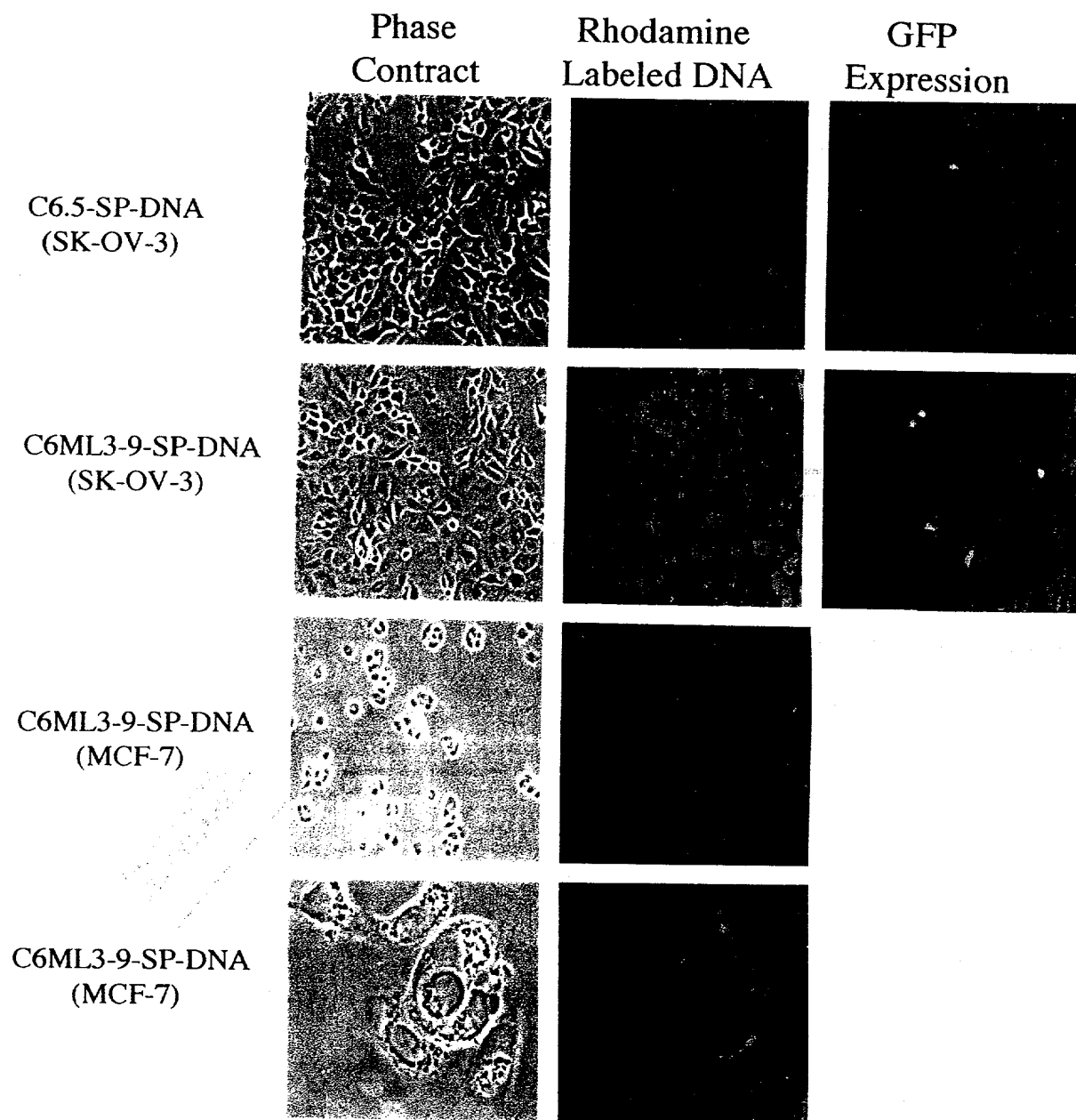


FIG. 21

THE EFFECT OF
CHLOROQUINE ON 3T3-HER2 TRANSFECTION
MEDIATED BY C6ML3-9sFv'-SALMON PROTAMINE

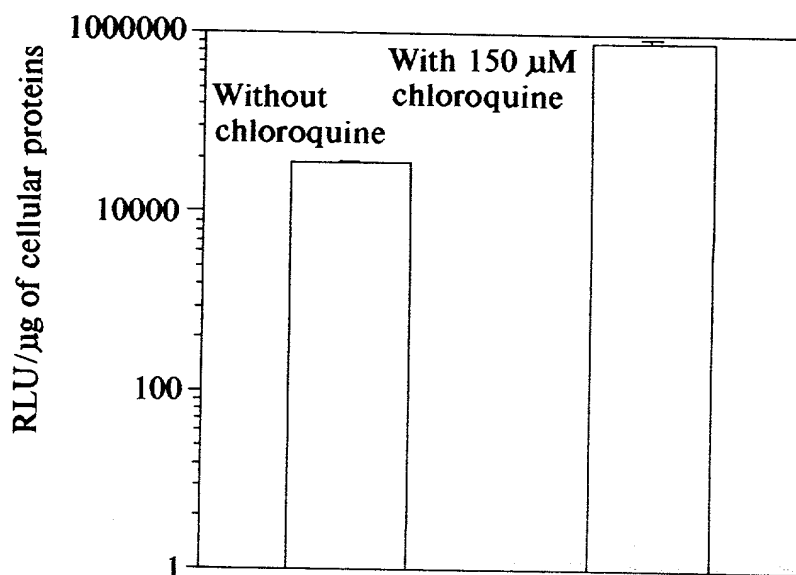


FIG. 22

THE EFFECT OF CHLOROQUINE ON 3T3-HER2
TRANSFECTION MEDIATED BY C6ML3-9sFv'#2-P1

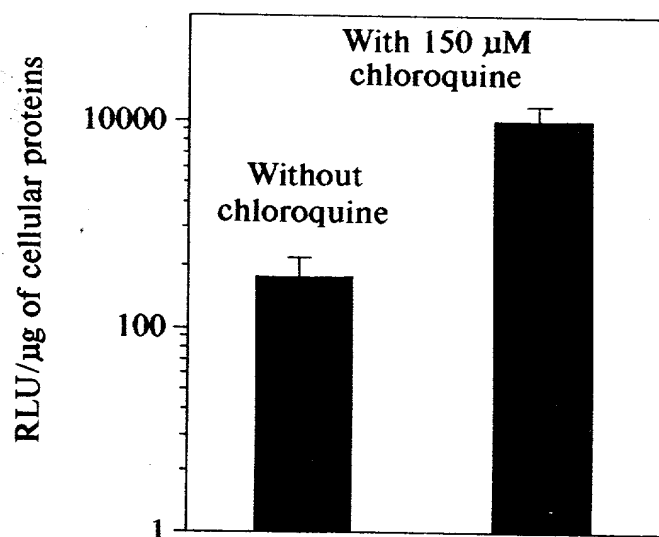


FIG. 23

17/18

THE EFFECT OF CHLOROQUINE ON 3T3-HER2 TRANSFECTION MEDIATED BY C6ML3-9sFv'#2-H1

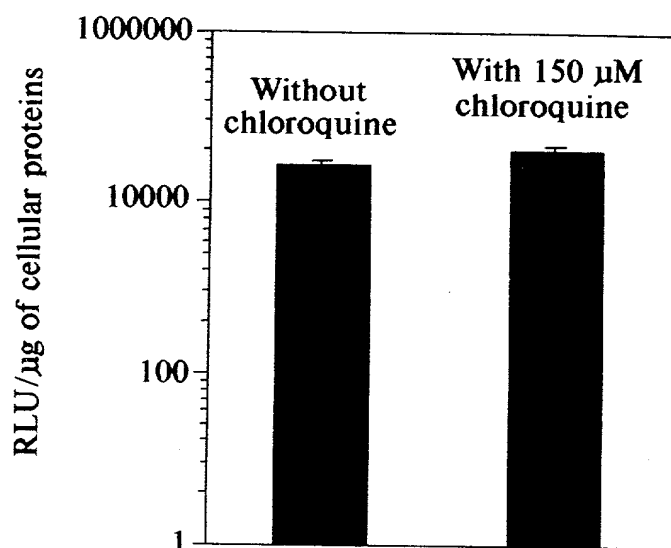


FIG. 24

THE EFFECT OF C6ML3-9sFv'-H1-pBks ON 3T3-HER2 TRANSFECTION MEDIATED BY C6ML3-9sFv'-H1

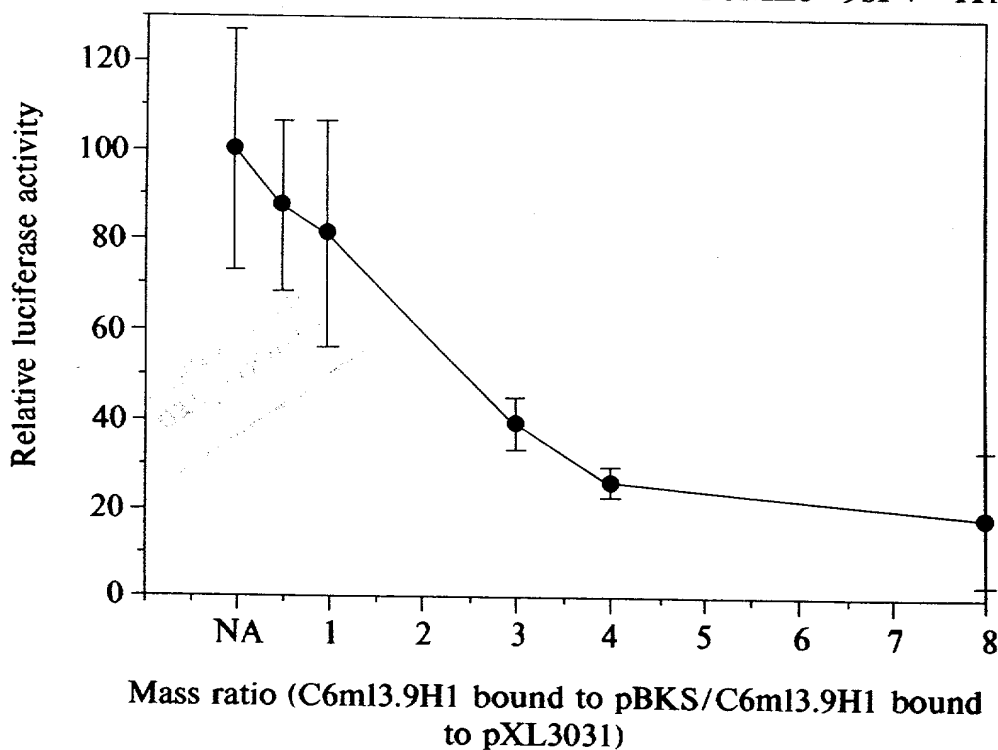


FIG. 25

THE EFFECT OF THE DNA TO C6ML3-9sFv'-H1
RATIO ON 3T3-HER2 TRANSFECTION EFFICIENCY

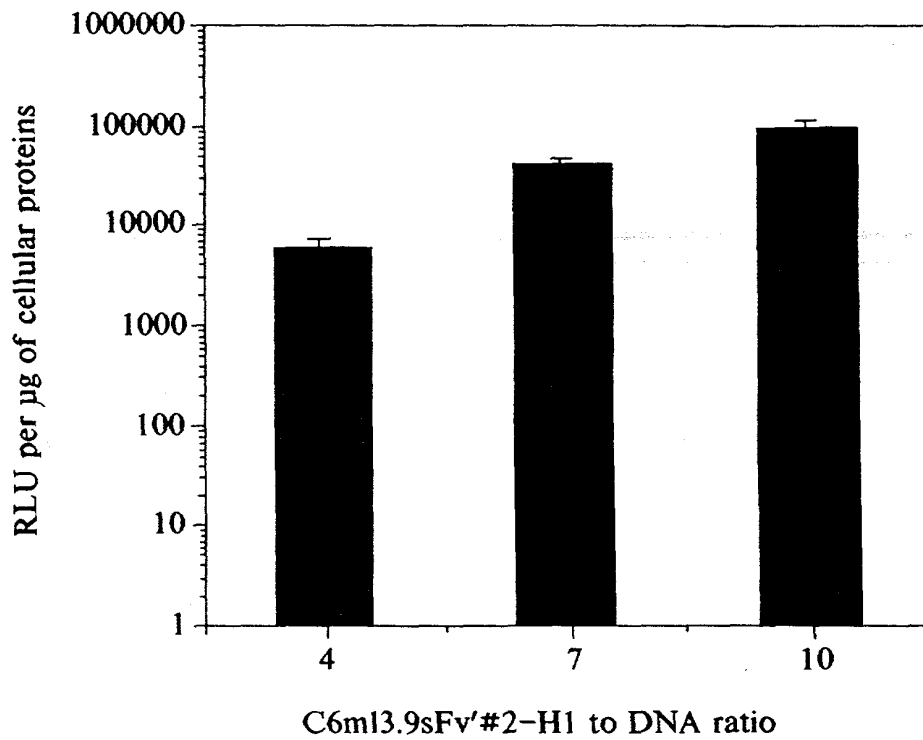


FIG. 26